# In the name of God

# The role of MicroRNAs in regeneration medicine

Presented by Zahra Jafari

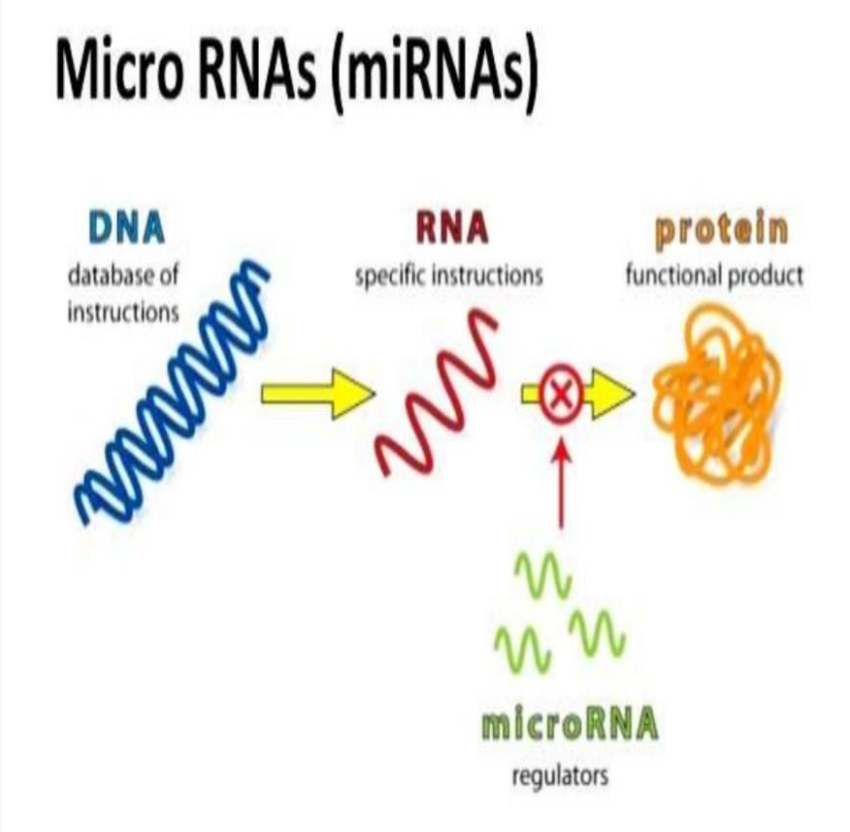
Under supervision of Dr.qeibi

01/29/2018

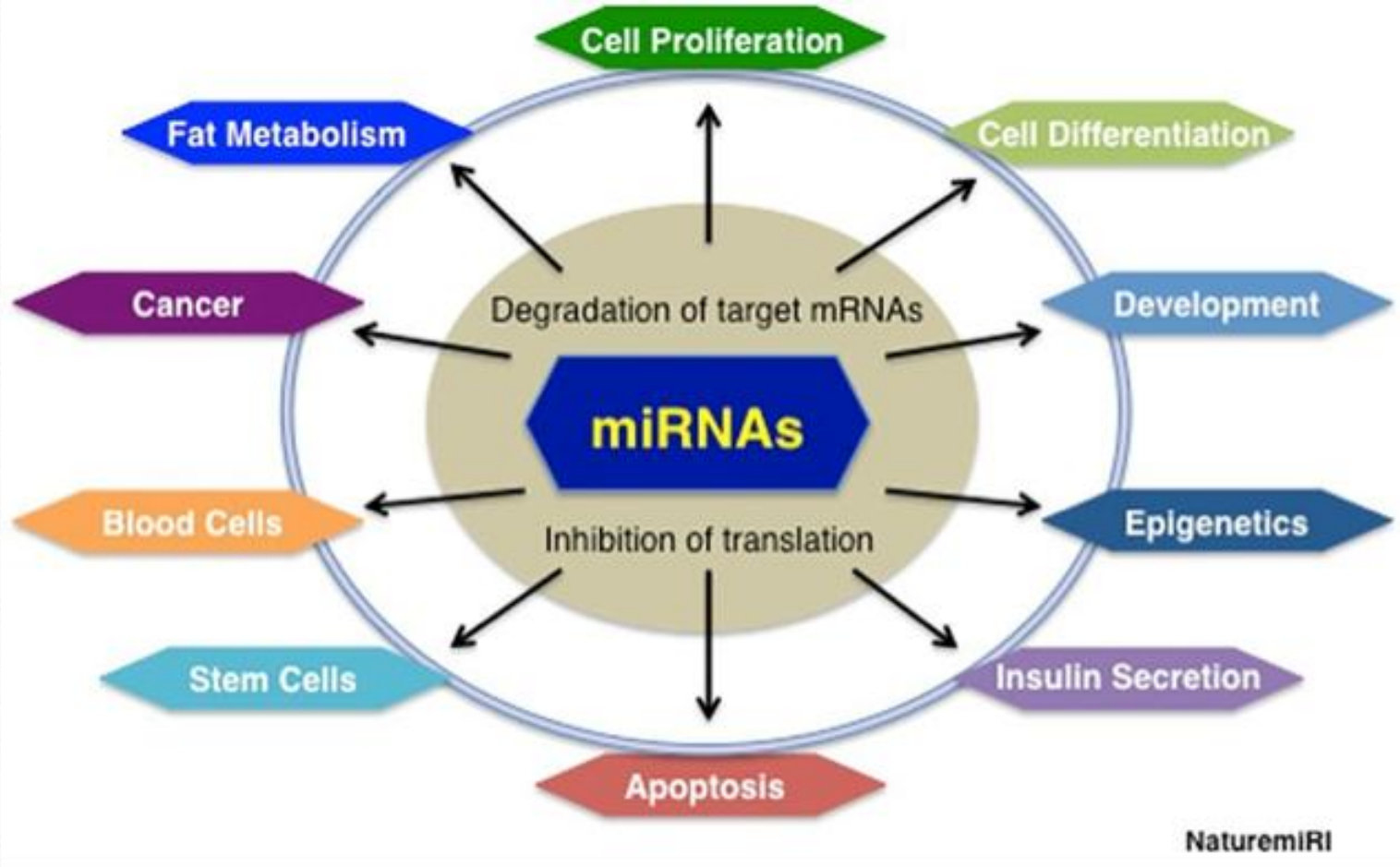
# Content

# What Are MicroRNAs?

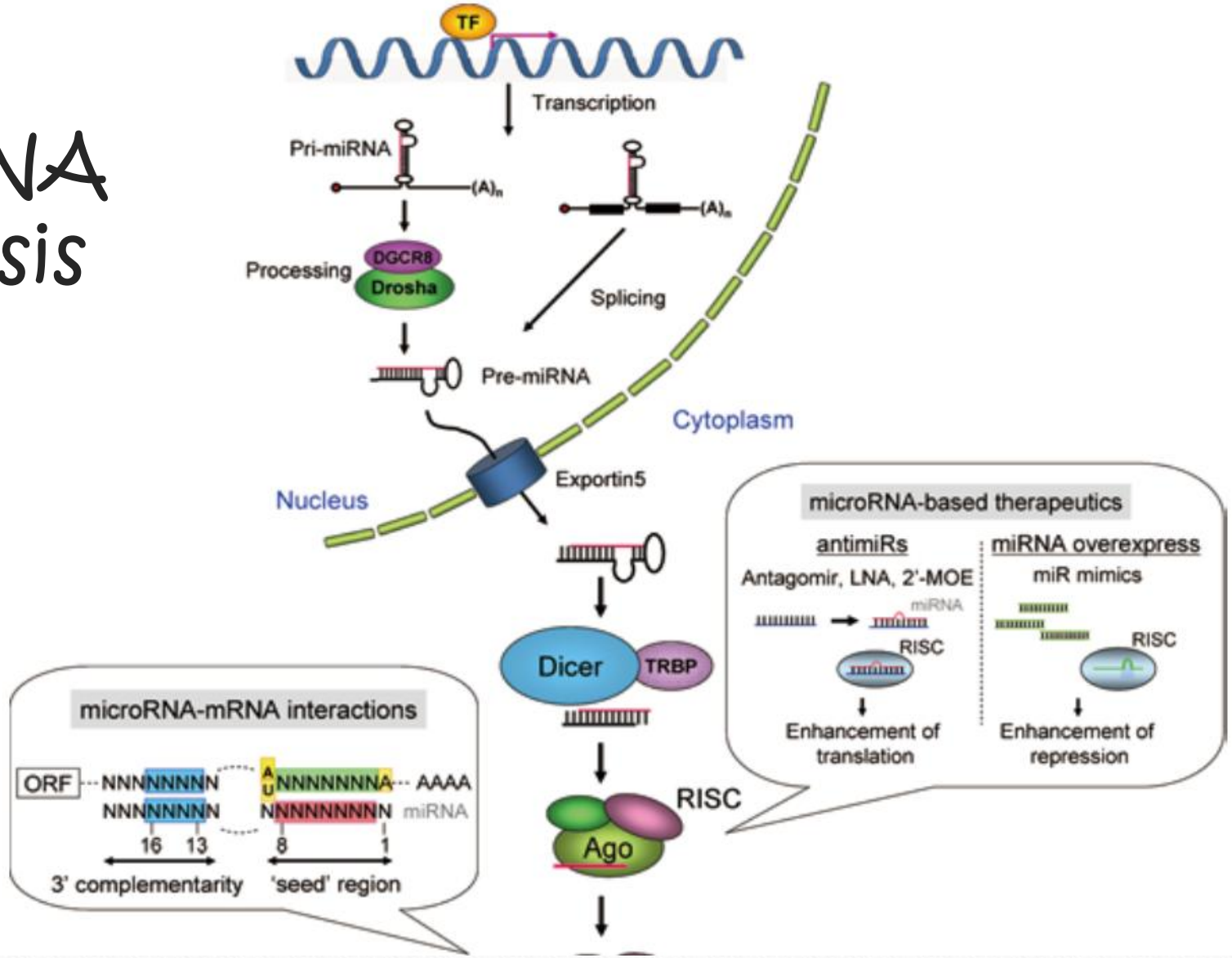
- 21 to 24 non-coding nucleotides
- that regulate gene expression by hybridizing to messenger RNAs (mRNAs)
- mRNA degradation or translational inhibition
- According to the **miRbase database** the human genome encodes **1,048** miRNAs



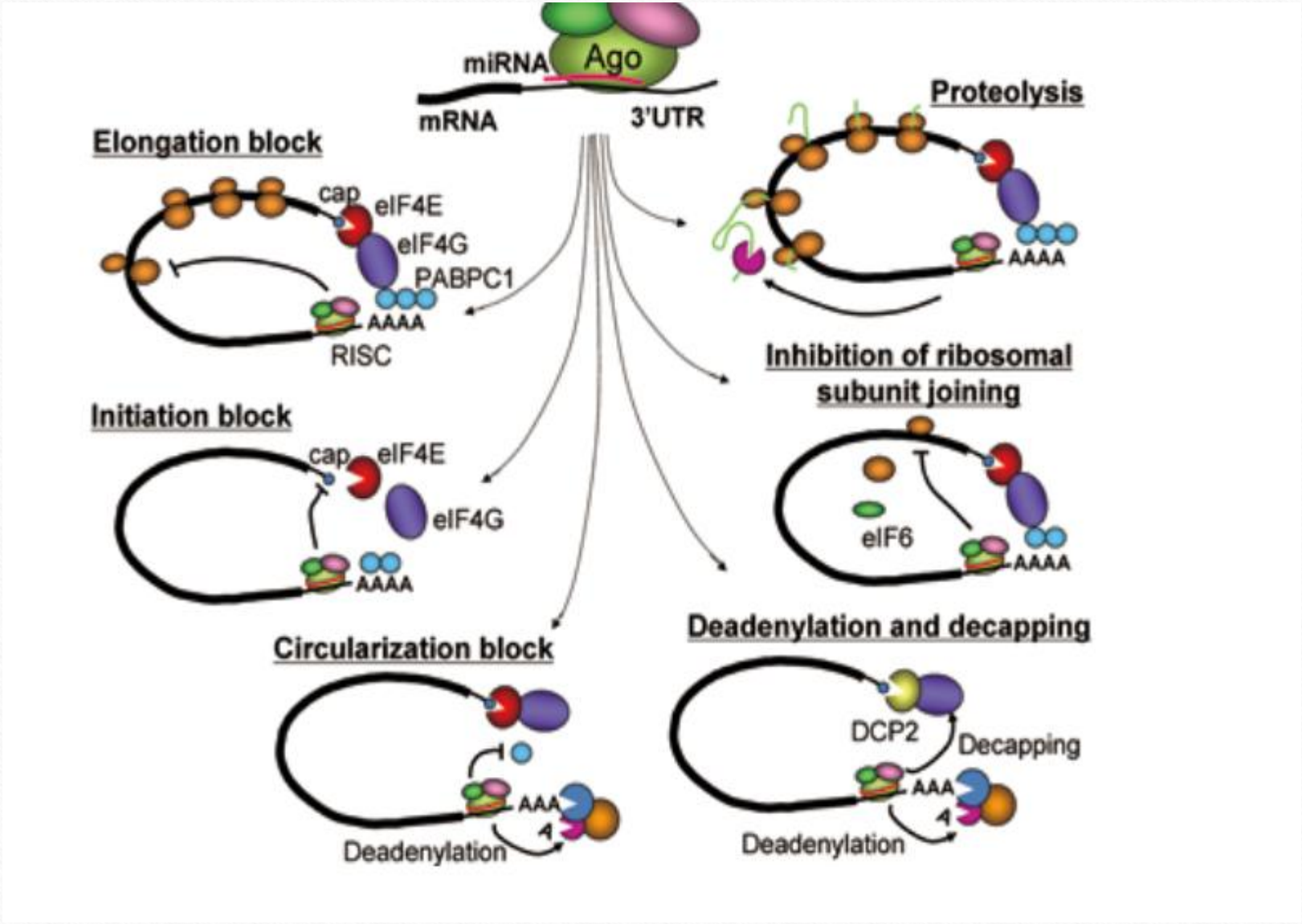
# The roles of microRNAs



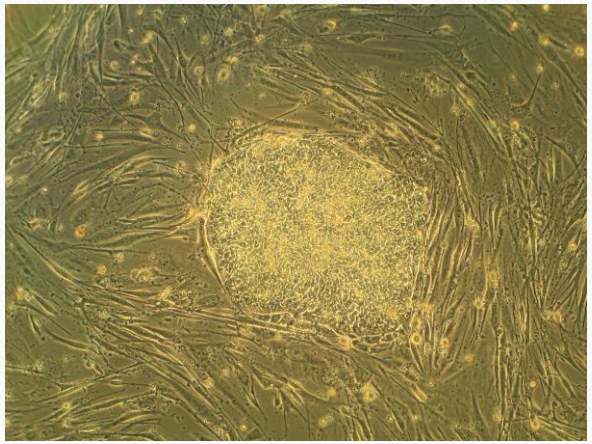
# MicroRNA biogenesis



# Repression Mechanisms of MicroRNAs



# Regenerative medicine



- is a branch of translational research
- in tissue engineering and molecular biology which
- deals with the "process of replacing, engineering or
- regenerating human cells, tissues or organs to restore or establish normal function

# miRNA Inhibition in Tissue Engineering and Regenerative Medicine

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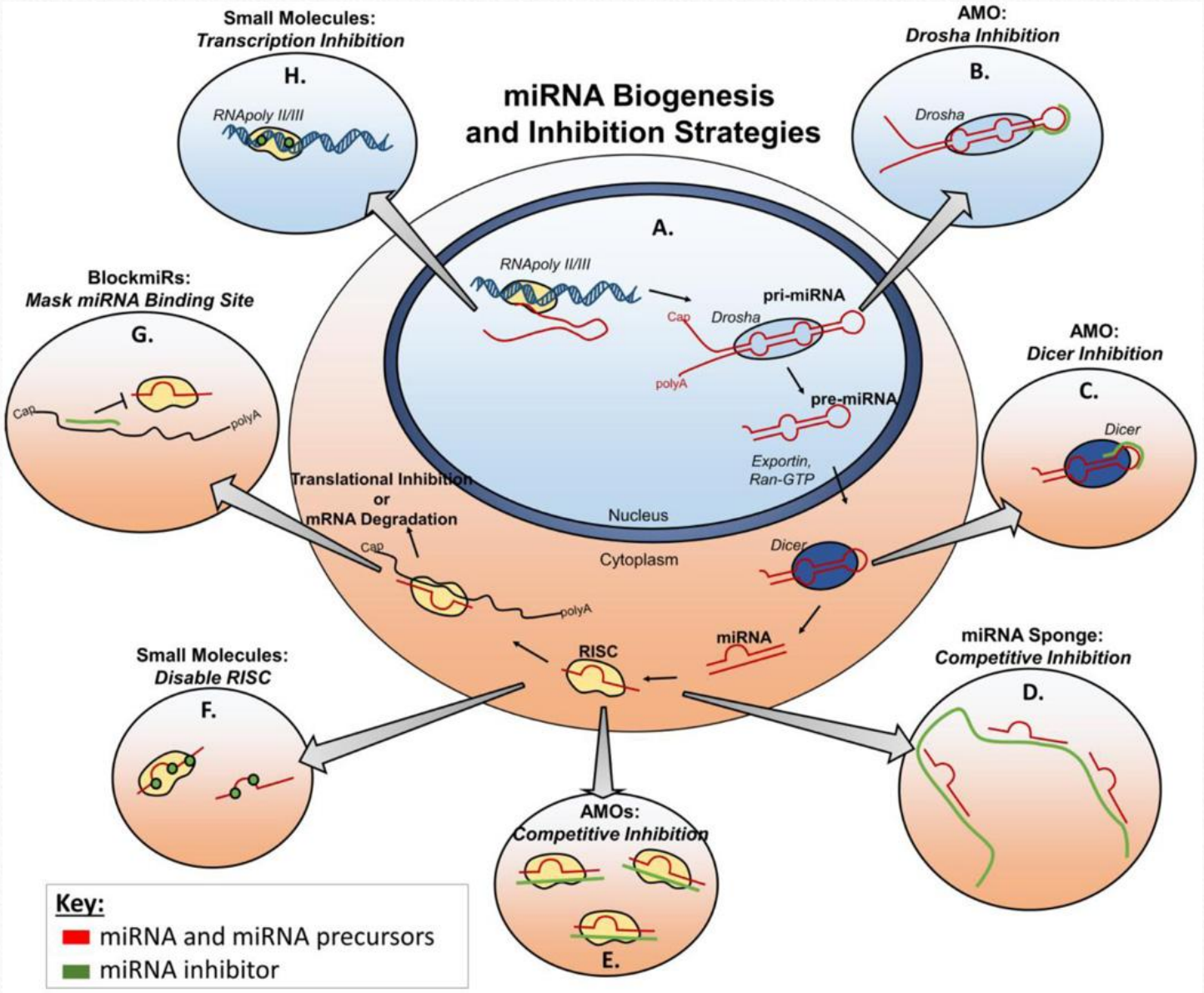
## Abstract

MicroRNA (miRNA) are noncoding RNA that provide an endogenous negative feedback mechanism for translation of messenger RNA (mRNA) into protein. Single miRNAs can regulate hundreds of mRNAs, enabling miRNAs to orchestrate robust biological responses by simultaneously impacting multiple gene networks. MiRNAs can act as master regulators of normal and pathological tissue development, homeostasis, and repair, which has recently motivated expanding efforts toward development of technologies for therapeutically modulating miRNA activity for regenerative medicine and tissue engineering applications. This review highlights the tools currently available for miRNA inhibition and their recent therapeutic applications for improving tissue repair.


miRNA Biogenesis
and Inhibition Strategies
Small Molecules:
Transcription Inhibition
H.
RNApoly II/III
AMO:
Drosha Inhibition
B.
Drosha
A.
RNApoly II/III
pri-miRNA
Cap
Drosha
polyA
pre-miRNA
Exportin,
Ran-GTP
Nucleus
Cytoplasm
BlockmiRs:
Mask miRNA Binding Site
G.
Cap
polyA
Translational Inhibition
or
mRNA Degradation
Cap
polyA
AMO:
Dicer Inhibition
C.
Dicer
Dicer
miRNA
RISC
Small Molecules:
Disable RISC
F.
AMOs:
Competitive Inhibition
E.
miRNA Sponge:
Competitive Inhibition
D.
Key:
miRNA and miRNA precursors
miRNA inhibitor

# Delivery consideration for anti- miRs

- ✓ **Viral delivery**
- ✓ **Non viral delivery**
- ✓ **Scaffold mediated delivery**

# Therapeutic application of anti miRs

## Potential Anti-miR Targets in Regenerative Medicine

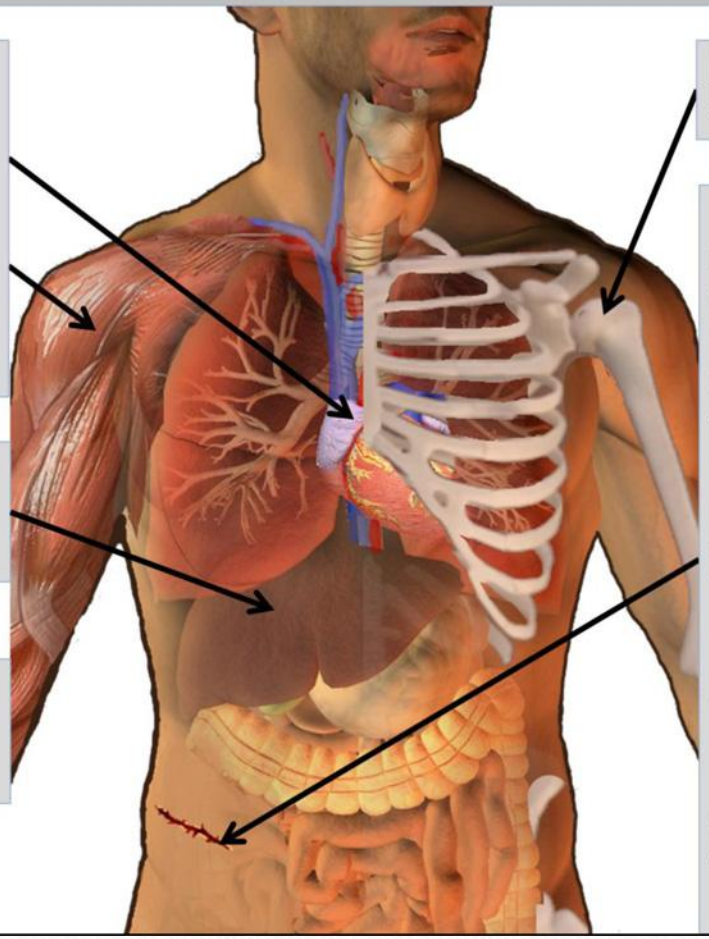
**Muscle Regeneration**

**Cardiac**
- miR-15, 25, 25, 92a

**Skeletal**
- miR-1, 92a, 203, and 206

**Liver Regeneration**
- miR-34a, 127, and 378

**Kidney Regeneration**
- miR-192



**Bone Regeneration**
- miR-31, and 92a

**Wound Healing**

**Inflammation:**
- Let-7a, miR-155, 221, 466l

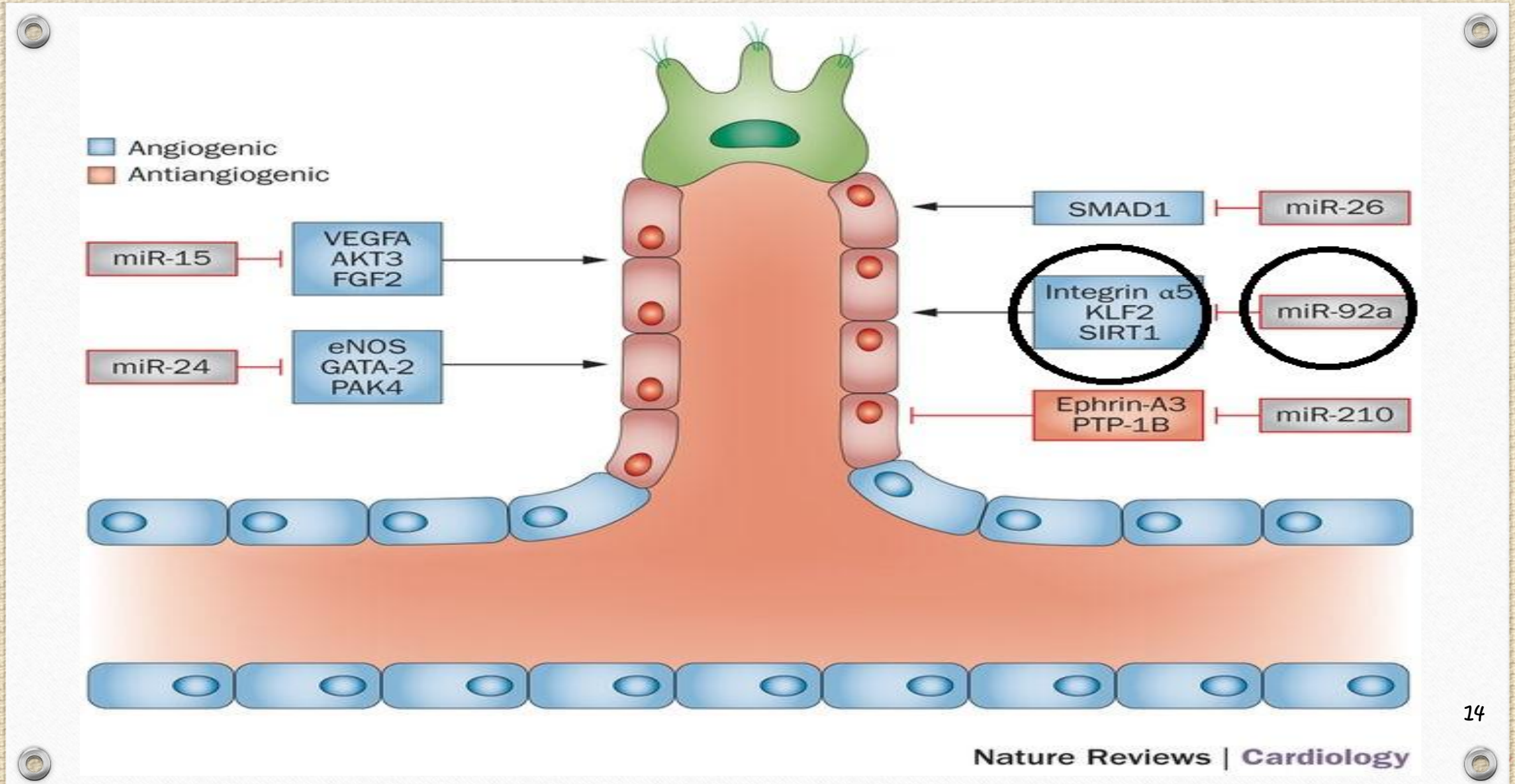
**Proliferation**
- miR-146a, 198, miR-200b, 210, 378a

**Angiogenesis**
- miR 17-92 cluster (17, 18a 19 a/b, 20a, 92a)
- miR-200b, 210, 221, 222, 320, and 503

**Scarless Healing**
- miR-1, 21, 24, 29, and 155

# Bone regeneration

- modulation of specific miRNA might allow for optimized bone regeneration
- miR-92a was down-regulated in human patients in response to trochanteric fractures
- MiR-92a targets integrin alpha-5 and mitogen-activated protein kinase 4
- So MiR 92a is inhibitor of angiogenesis


Angiogenic
Antiangiogenic
miR-15
VEGFA
AKT3
FGF2
miR-24
eNOS
GATA-2
PAK4
SMAD1
miR-26
Integrin α5
KLF2
SIRT1
miR-92a
Ephrin-A3
PTP-1B
miR-210
Nature Reviews | Cardiology

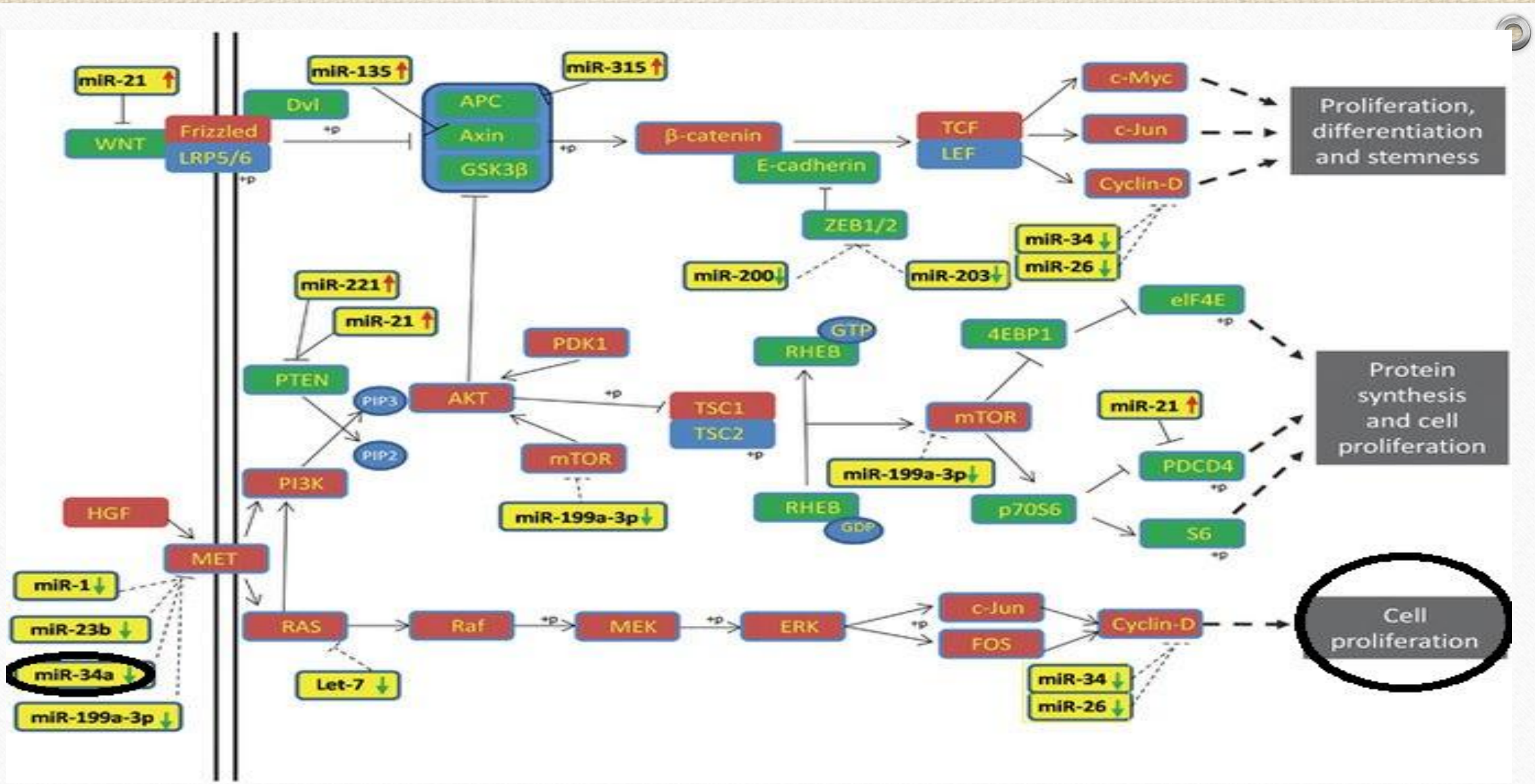
# Liver regeneration

- **Liver is an auto repair organ**
- **Many studies have shown that miRNA are**

**key regulators of hepatic regeneration process, including**

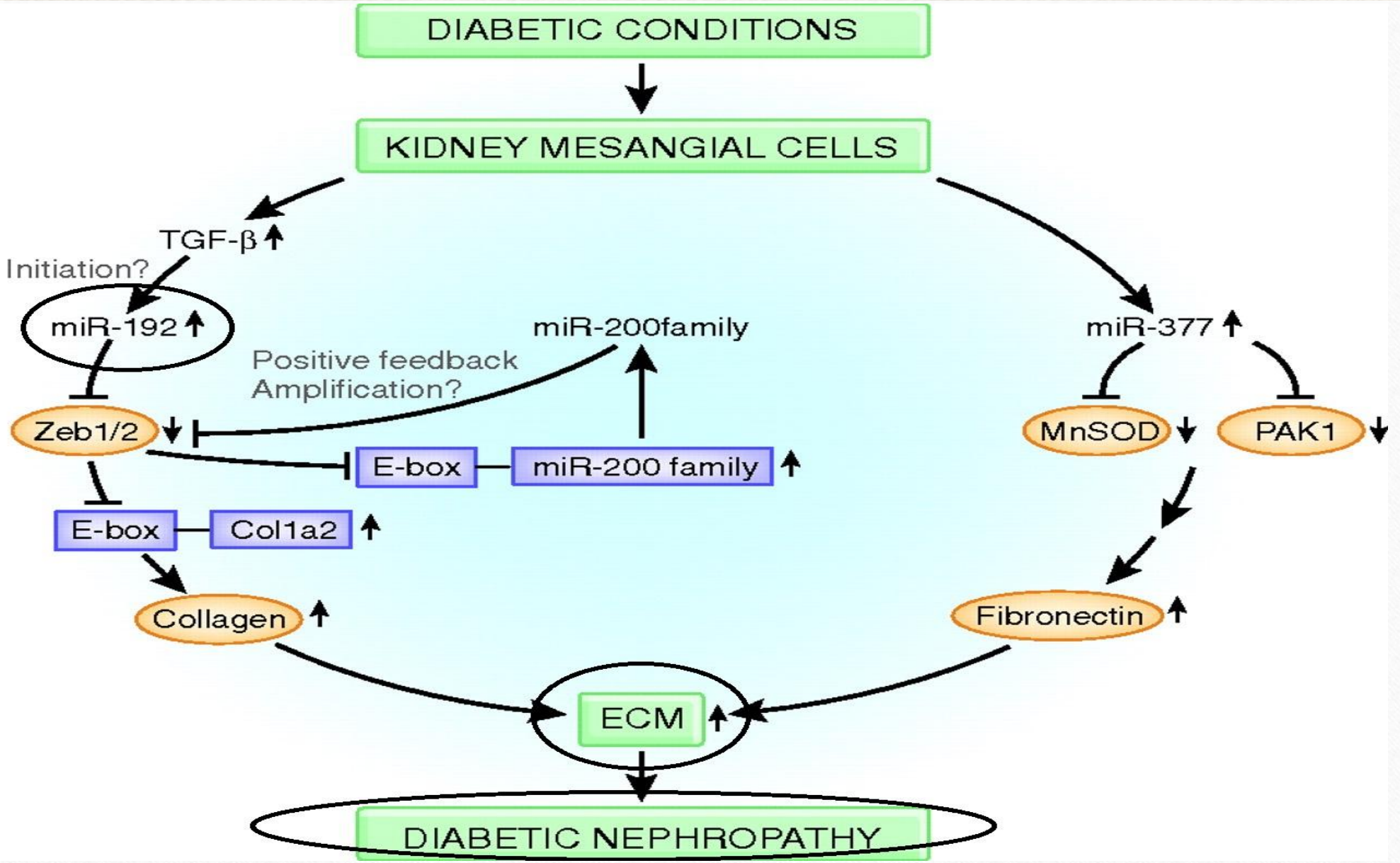
**miR-378, 127, and 34a.**

- **Increasing Mir 378 promotes DNA synthesis and target ornithine decarboxylase**
- **Decreasing Mir 127 increase hepatocyte proliferation**
- **up-regulation of miR-34a suppresses hepatocyte proliferation**


miR-21
WNT
Frizzled
LRP5/6
Dvl
miR-135
APC
Axin
GSK3β
miR-315
β-catenin
E-cadherin
TCF
LEF
c-Myc
c-Jun
Cyclin-D
Proliferation, differentiation and stemness
ZEB1/2
miR-200
miR-203
miR-34
miR-26
miR-221
miR-21
PTEN
PIP3
PIP2
PI3K
AKT
PDK1
mTOR
miR-199a-3p
TSC1
TSC2
GTP
RHEB
GDP
RHEB
mTOR
miR-199a-3p
4EBP1
eIF4E
p70S6
PDCD4
S6
miR-21
Protein synthesis and cell proliferation
HGF
MET
miR-1
miR-23b
miR-34a
miR-199a-3p
RAS
Raf
MEK
ERK
Let-7
c-Jun
FOS
Cyclin-D
miR-34
miR-26
Cell proliferation

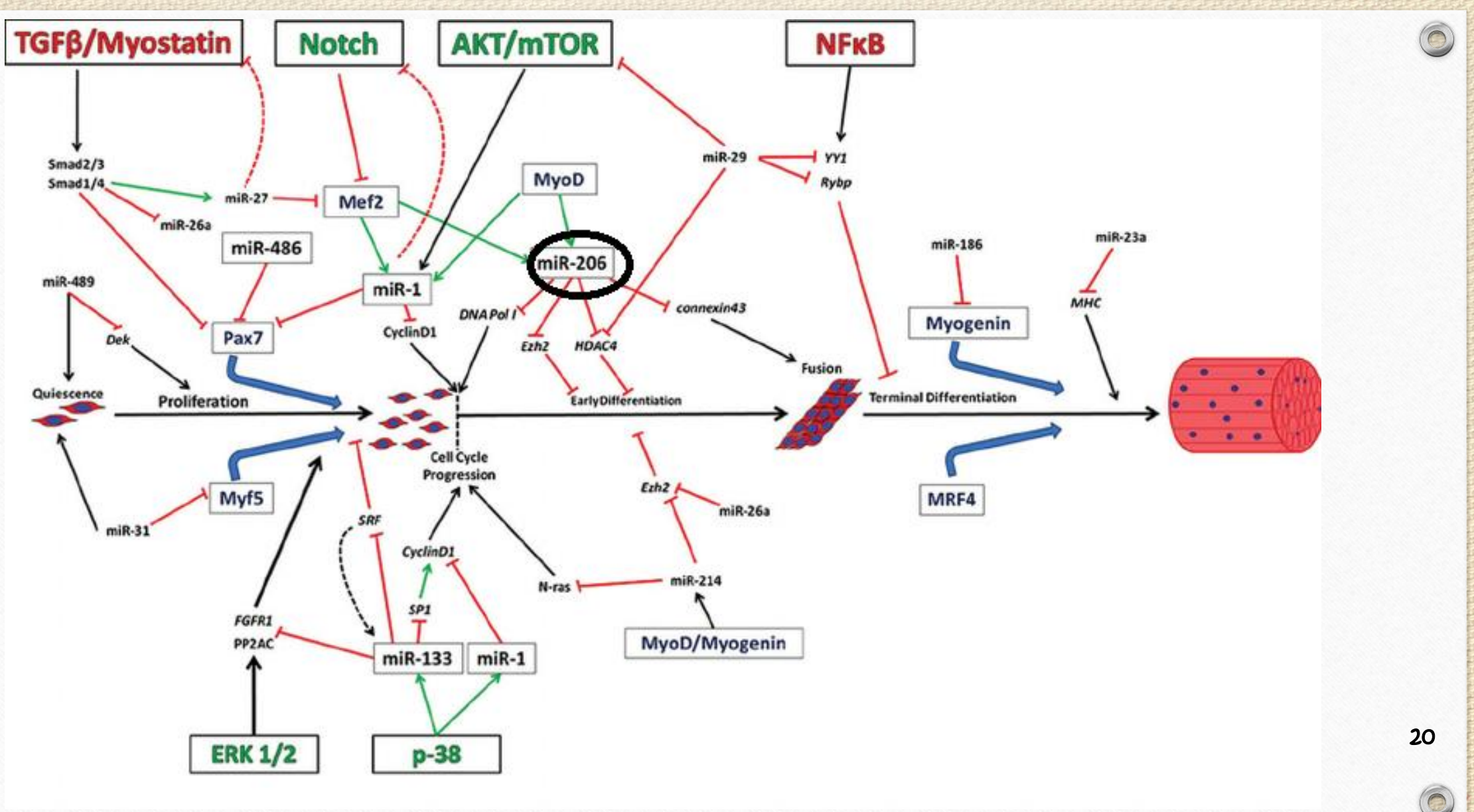
# Kidney regeneration

- **cellular repair through mitosis and proliferation of neighboring cells**
- **pro- angiogenic miRNAs released from endothelial progenitor cells following renal ischemia-reperfusion injury**
- **possibility could be to culture stem cells *ex vivo* for reprogramming and deliver them to damaged kidneys**
- **Diabetic nephropathy (DN) is a chronic, incurable disease that is the most common cause of total renal failure**
- **miR-192 as a potential target for inhibition in order to decrease the collagen accumulation associated with DN fibrosis**


DIABETIC CONDITIONS
KIDNEY MESANGIAL CELLS
TGF-β
Initiation?
miR-192
miR-200family
Positive feedback
Amplification?
Zeb1/2
E-box
miR-200 family
E-box
Col1a2
Collagen
miR-377
MnSOD
PAK1
Fibronectin
ECM
DIABETIC NEPHROPATHY

# Muscle regeneration

- **miR-1, miR-206, and miR-133 are responsible for muscle differentiation**
- **presence of miR-206 improves skeletal muscle regeneration in Duchenne muscular dystrophy**
- **Mir 203 inhibit skeletal muscle proliferation and differentiation by inhibiting c-Jun and MEF2C and may be a potential therapeutic target for anti-miRs**


TGFβ/Myostatin
Notch
AKT/mTOR
NFκB
Smad2/3
Smad1/4
miR-27
miR-26a
Mef2
MyoD
miR-29
YY1
Rybp
miR-486
miR-206
miR-186
miR-23a
miR-489
miR-1
Myogenin
MHC
Dek
Pax7
CyclinD1
DNA Pol I
connexin43
Ezh2
HDAC4
Fusion
Quiescence
Proliferation
Early Differentiation
Terminal Differentiation
Cell Cycle Progression
Myf5
MRF4
miR-31
SRF
Ezh2
miR-26a
CyclinD1
N-ras
miR-214
SP1
FGFR1
PP2AC
miR-133
miR-1
MyoD/Myogenin
ERK 1/2
p-38

ATVB in Focus
MicroRNAs: From Basic Mechanisms to Clinical Application in Cardiovascular Medicine

Series Editor: Christian Weber

# MicroRNAs in Stem Cell Function and Regenerative Therapy of the Heart

Florian H. Seeger, Andreas M. Zeiher, Stefanie Dimmeler

***Abstract*—MicroRNAs** are small noncoding RNAs that posttranscriptionally control gene expression by targeting mRNAs. Distinct microRNAs regulate stem and progenitor cell functions, thereby modulating cell survival and homing or controlling differentiation and maturation. Experimental studies additionally show that microRNAs regulate endogenous repair and might potentially be useful to enhance the regeneration of the heart. This review summarizes the current studies that address the use of microRNAs to either improve cellular therapies or that might be targeted for enhancing endogenous tissue repair and regeneration after myocardial infarction. (***Arterioscler Thromb Vasc Biol*. 2013;33:1739-1746.)**

**Key Words:** cardiac repair ■ miR ■ microRNA ■ regenerative therapy ■ stem cell function

# Cardiac regeneration

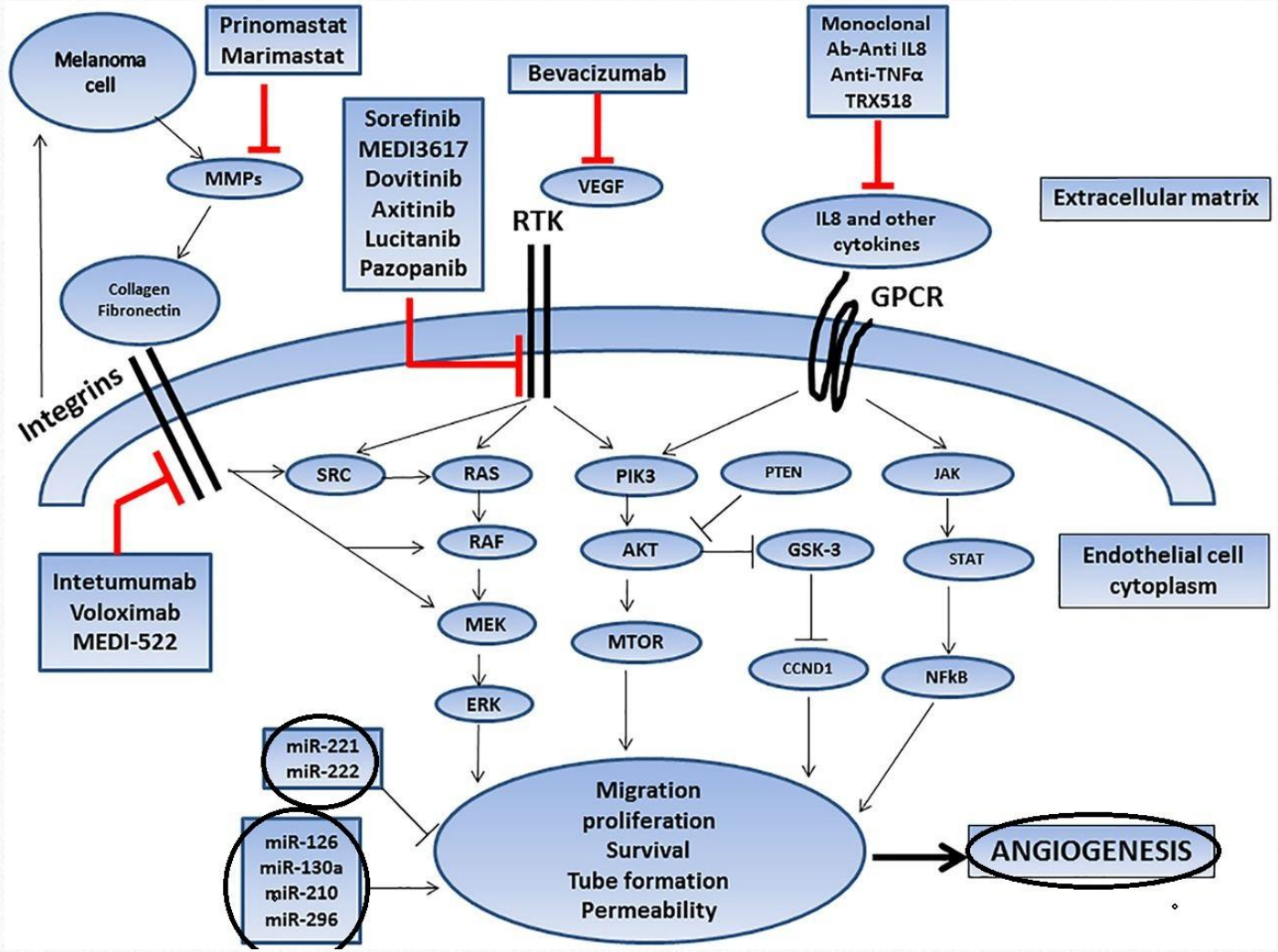
- After an acute myocardial infarction, the death of cardio myocytes , Cannot replaced by endogenous regeneration
- the heart susceptible for unfavorable remodeling and heart failure
- adult stem/progenitor cells
- bone marrow–derived proangiogenic cells
- mesenchymal stromal cells were shown recovery after ischemia.
- Embryonic or induced pluripotent stem cells have shown

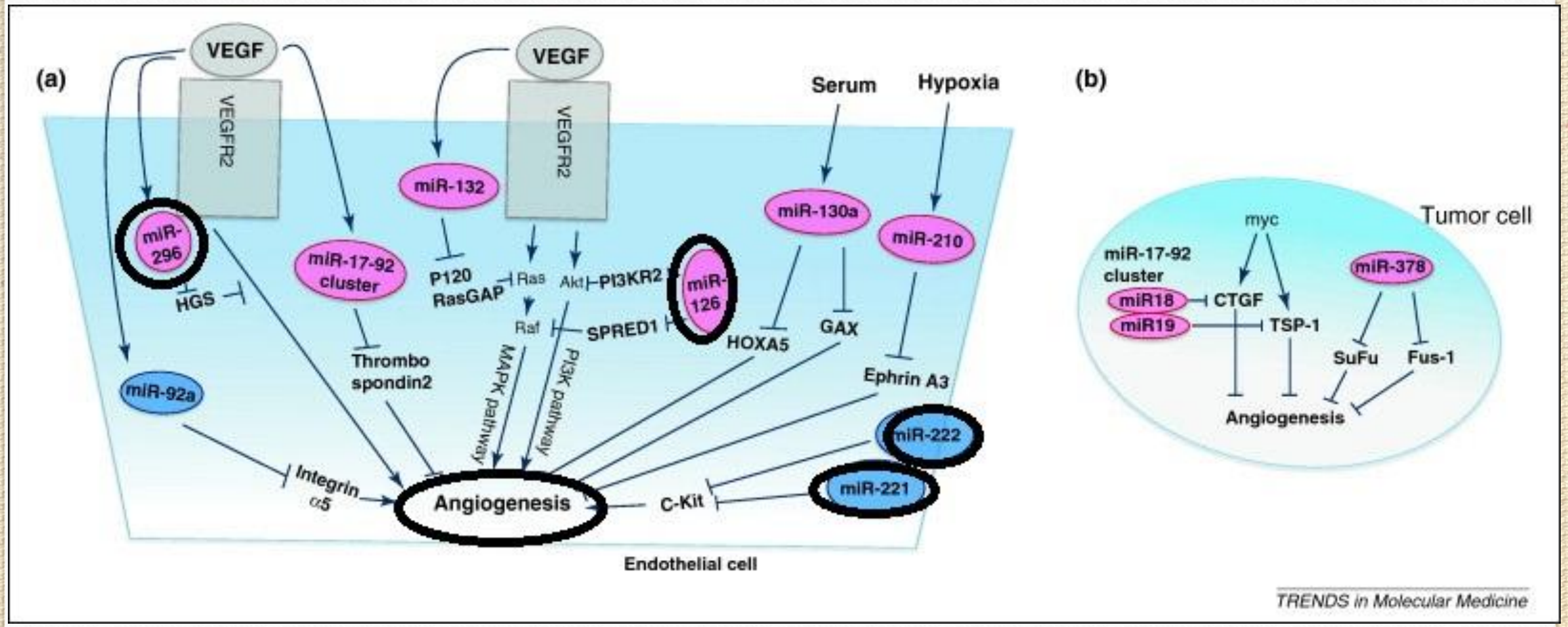
the highest capacity to replace dead myocardium

Myocardial infarction, profoundly disturbs the expression of miRNAs

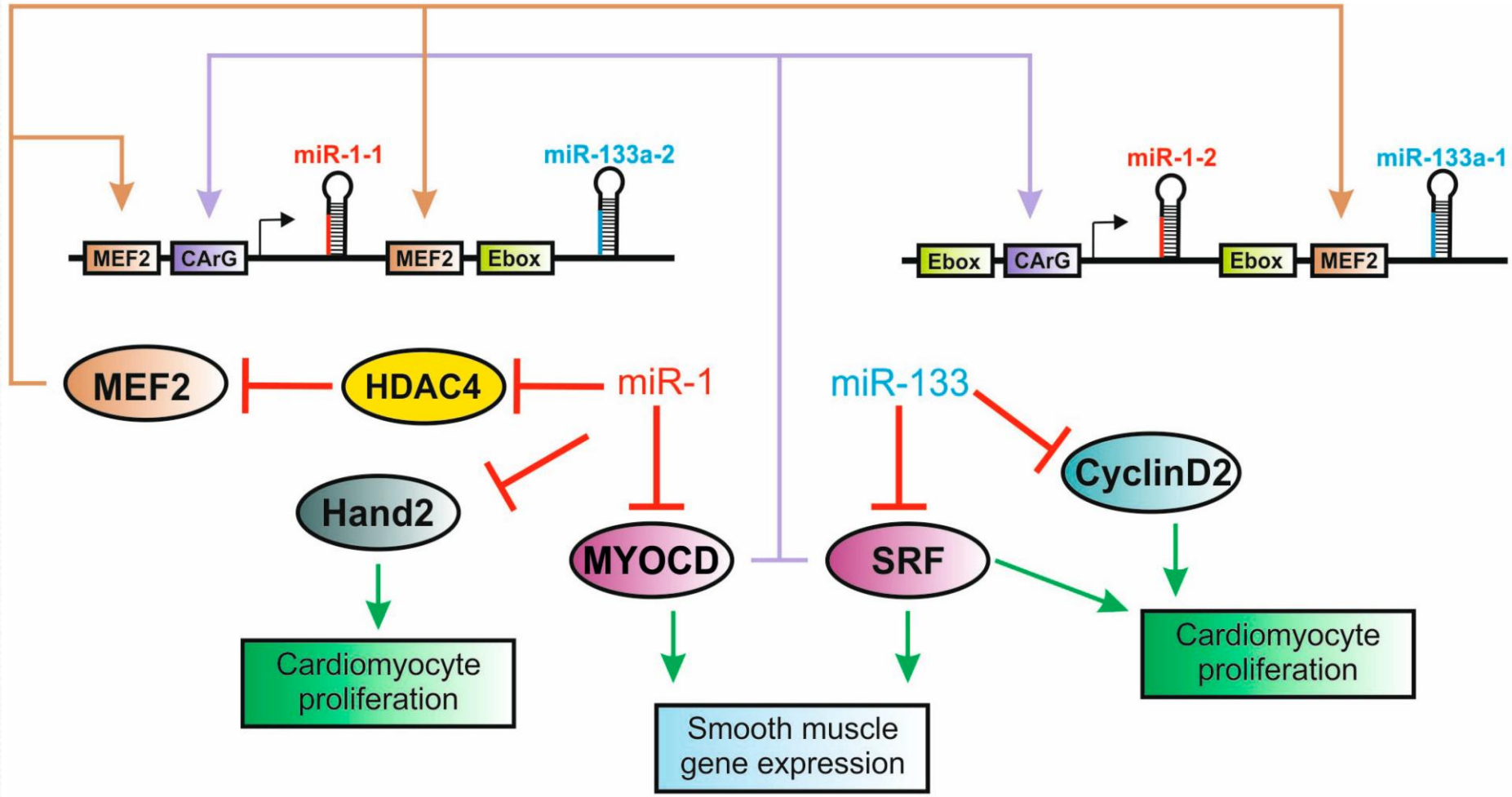
- MiRNAs also have an important influence on stem cells

# Mir 126-mir 296-mir 101-mir 221-mir 24-mir 21-mir155-mir 34a




(a)
VEGF
VEGFR2
VEGF
VEGFR2
Serum
Hypoxia
miR-296
HGS
miR-17-92 cluster
Thrombo spondin2
miR-92a
Integrin α5
miR-132
P120 RasGAP
Ras
Raf
MAPK pathway
Akt
PI3KR2
PI3K pathway
miR-126
SPRED1
miR-130a
HOXA5
GAX
miR-210
Ephrin A3
miR-222
miR-221
C-Kit
Angiogenesis
Endothelial cell
(b)
Tumor cell
myc
miR-17-92 cluster
miR18
miR19
CTGF
TSP-1
miR-378
SuFu
Fus-1
Angiogenesis
TRENDS in Molecular Medicine

# Mir 1 –mir133 in cardiac regeneration

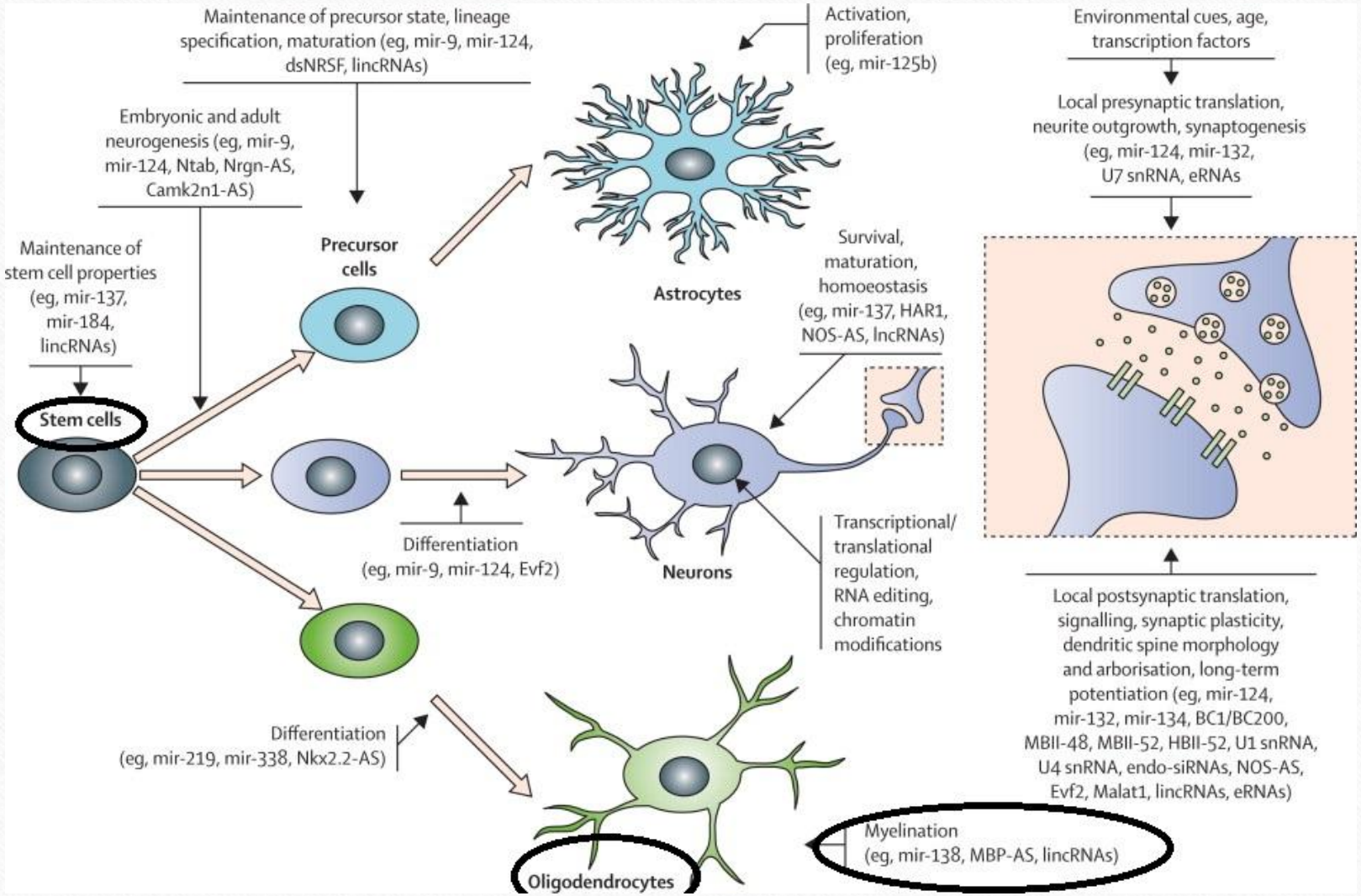


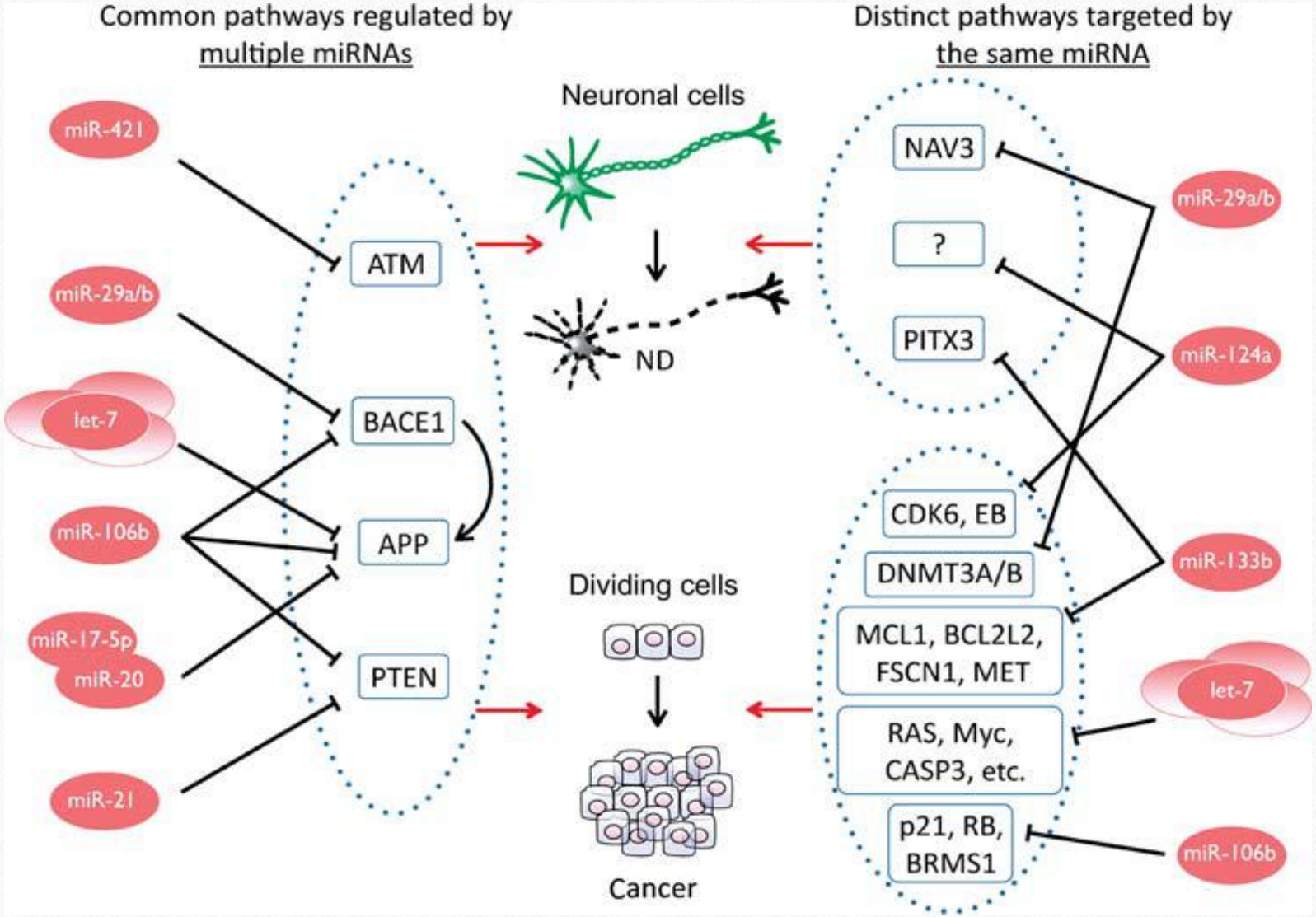
# Wound healing

- Wound healing requires
- sequence of stages known as coagulation, inflammation, proliferation, angiogenesis maturation, and remodeling, which are tightly regulated by miRNAs
- Mir 155-let7a-mir221-mir4661 in inflammation
- Mir378a-mir200-mir210-mi198 in proliferation
- Mir221/222-320-200b-mir 503mir17-mir20 in angiogenesis

# The roles of Mir s in neuroregenerative medicine

Mir 21-MBPs




Common pathways regulated by
multiple miRNAs
Distinct pathways targeted by
the same miRNA
Neuronal cells
miR-421
miR-29a/b
let-7
miR-106b
miR-17-5p
miR-20
miR-21
ATM
BACE1
APP
PTEN
ND
NAV3
?
PITX3
CDK6, EB
DNMT3A/B
MCL1, BCL2L2,
FSCN1, MET
RAS, Myc,
CASP3, etc.
p21, RB,
BRMS1
miR-29a/b
miR-124a
miR-133b
let-7
miR-106b
Dividing cells
Cancer

# Reprogramming of cells to pluripotency and using MiR

## Reprogramming of Mouse and Human Cells to Pluripotency Using Mature MicroRNAs

Norikatsu Miyoshi,[1] Hideshi Ishii,[1,2,4,*] Hiroaki Nagano,[1] Naotsugu Haraguchi,[1] Dyah Laksmi Dewi,[1] Yoshihiro Kano,[1] Shinpei Nishikawa,[1] Masahiro Tanemura,[1] Koshi Mimori,[2] Fumiaki Tanaka,[2] Toshiyuki Saito,[3] Junichi Nishimura,[1] Ichiro Takemasa,[1] Tsunekazu Mizushima,[1] Masataka Ikeda,[1] Hirofumi Yamamoto,[1] Mitsugu Sekimoto,[1] Yuichiro Doki,[1] and Masaki Mori[1,2,4,*]

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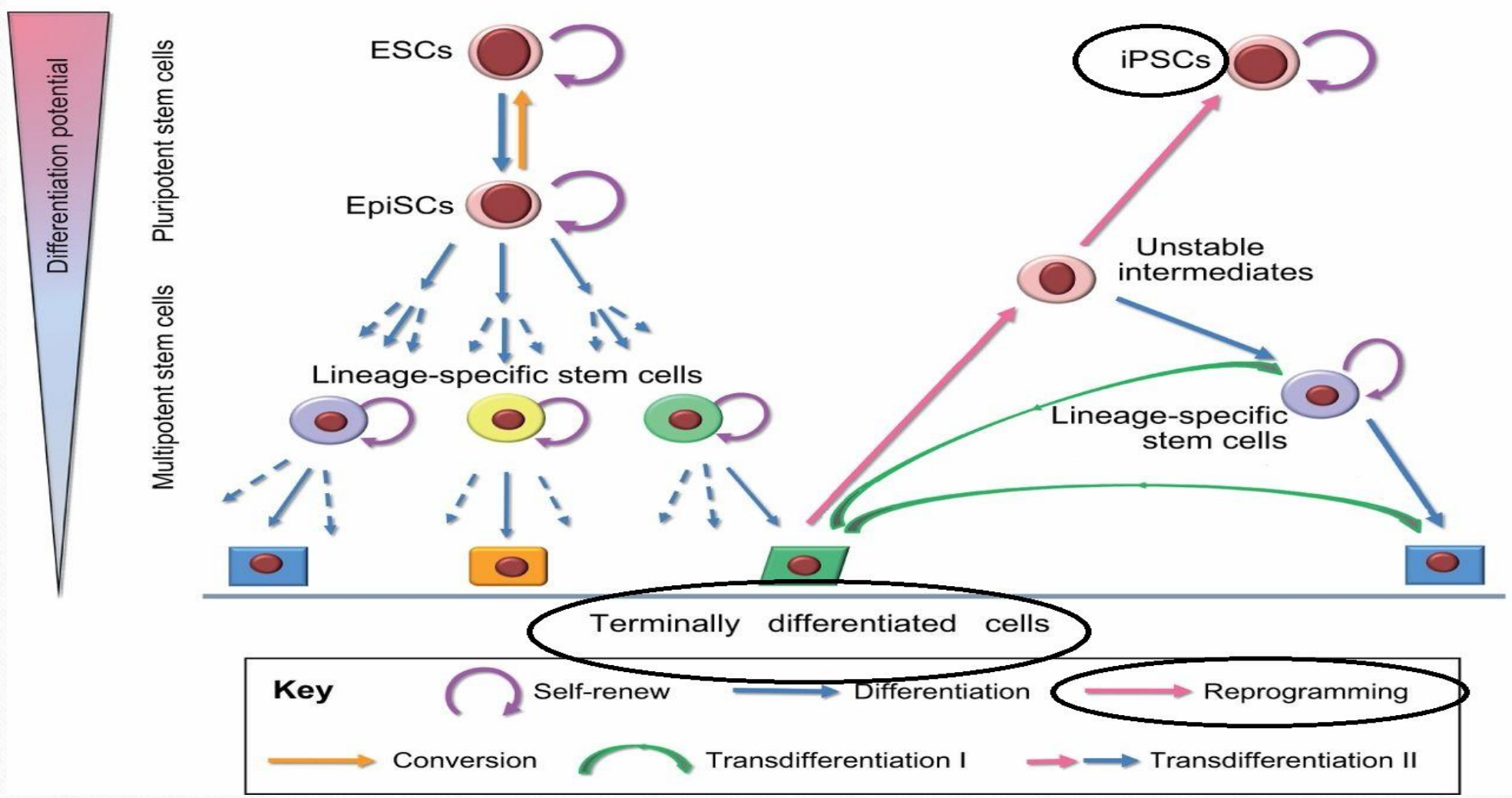
**SUMMARY**

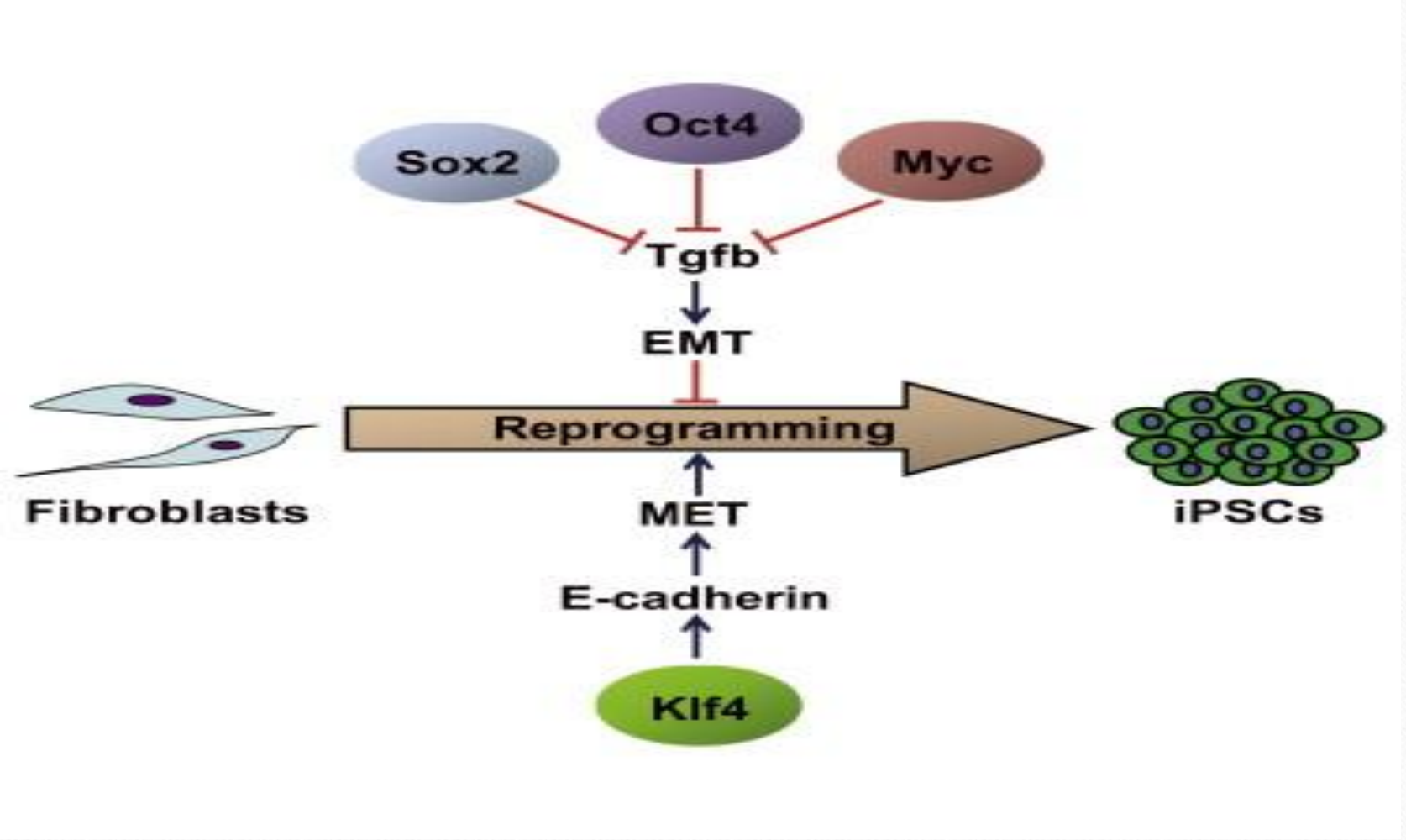
Induced pluripotent stem cells (iPSCs) can be gener-

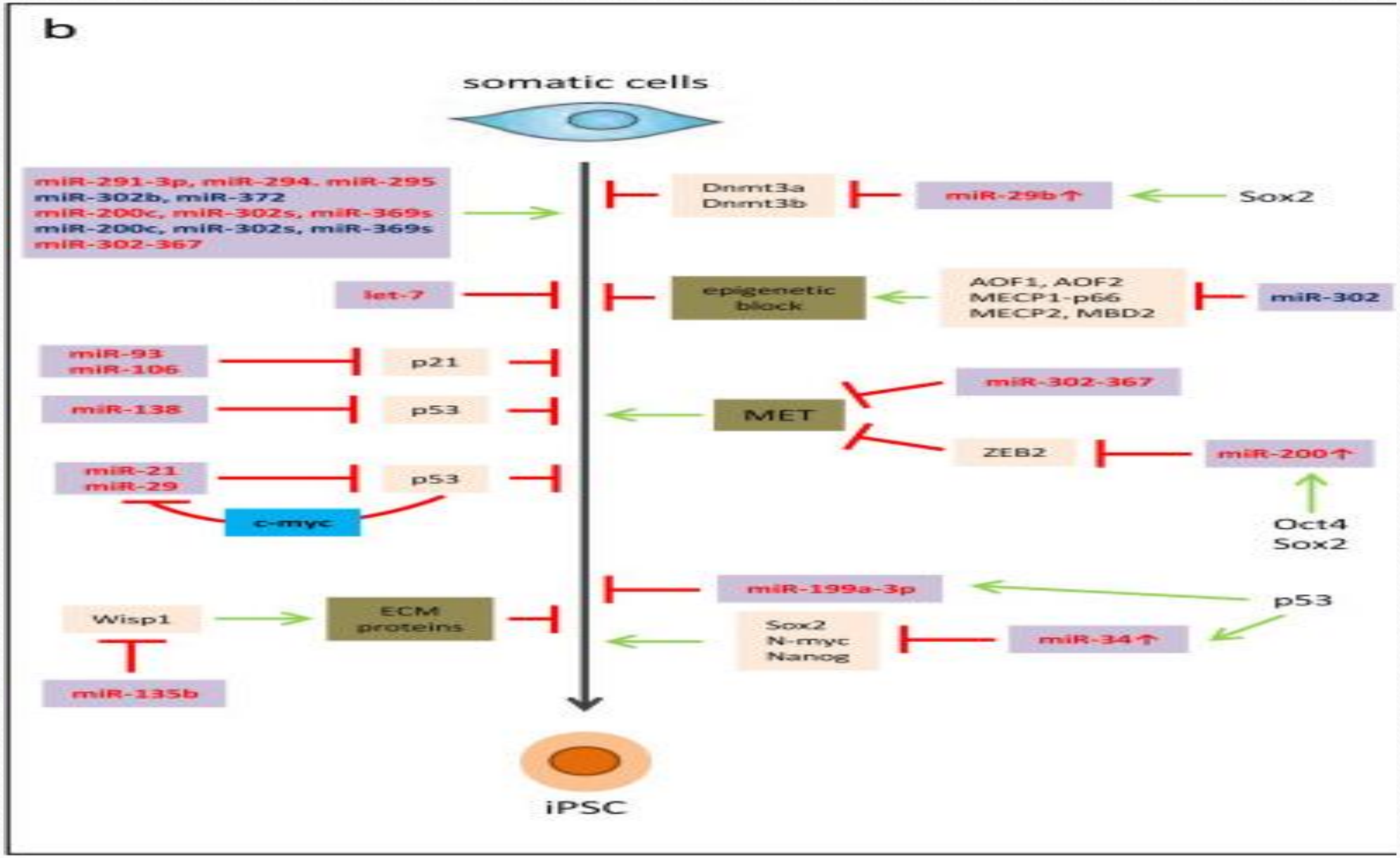
example, in Seki et al., 2010), because it has an RNA genome, but it is still a virus. Reprogramming using just protein or mRNA has also been reported, but the protocols involved are

# Reprogramming of cells to pluripotency and using Mir



# Reprogramming of cells to pluripotency and using Mir




b
somatic cells
miR-291-3p, miR-294, miR-295
miR-302b, miR-372
miR-200c, miR-302s, miR-369s
miR-200c, miR-302s, miR-369s
miR-302-367
Dnmt3a
Dnmt3b
miR-29b↑
Sox2
let-7
epigenetic block
AOF1, AOF2
MECP1-p66
MECP2, MBD2
miR-302
miR-93
miR-106
p21
miR-138
p53
MET
miR-302-367
ZEB2
miR-200↑
miR-21
miR-29
p53
c-myc
Oct4
Sox2
miR-199a-3p
p53
Wisp1
ECM proteins
Sox2
N-myc
Nanog
miR-34↑
miR-135b
iPSC

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